A reassessment of the role of simple seed traits in mortality following herbivore ingestion

Bram D'hondt, Maurice Hoffmann

In: Plant Biology, 2010

A reassessment of the role of simple seed traits in mortality following herbivore ingestion

B. D'HONDT[1] & M. HOFFMANN[1,2]

[1]*Terrestrial ecology unit, Biology department, Ghent university, Ghent, Belgium*

[2]*Research institute for nature and forest, Brussels, Belgium*

Running head:

Seed mortality following ingestion by herbivores

Correspondence:

B. D'hondt, Terrestrial ecology unit, Biology department, Ghent university, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

E-mail: b.dhondt@ugent.be

Keywords:

seed dispersal; endozoochory; zoochory; ungulates; foliage is the fruit; phylogenetically independent contrasts; hardseededness; physical dormancy

ABSTRACT

Grazing mammals are regarded as major vectors in seed dispersal of grassland plants through seed ingestion and subsequent excretion (endozoochory). The (evolutionary) ecology underlying this dispersal mode is understood relatively poorly as we have limited data, among others, on how seed attributes perform in this process (and could thus be selected for). Regarding mortality due to digestion, contrasting patterns found on the role of simple seed traits seem partly due to inadequate comparative-analytical methods. We conducted a feeding experiment in which controlled seed quantities from a large number of grassland herbs and grasses were fed to cattle (48 species in total). Seed mass, length and shape measurements were related to mortality rates using phylogenetically independent contrasts, which account for taxonomical interdependence. The proportion of seeds surviving the digestive tract was generally low, but ranged up to 100 %, with increased germination for some species. Neither seed size nor shape correlated significantly with mortality. Structural traits are likely to overrule these simple seed traits, as illustrated by high survival rates of species that are characterized by water-impermeable seeds. As the latter trait holds some interesting properties with respect to intraspecific variation and genetics, it could open up perspectives for inference on any evolutionary consequences for this type of dispersal.

INTRODUCTION

Plant propagules serve to ensure the continuation of populations through time and space. In nature, seeds – broadly defined as any propagules of generative origin (Cousens *et al.* 2008) – disperse via a vast range of mechanisms, involving both abiotic and biotic vectors. Among the latter, herbivorous mammals are regarded as a major group of dispersers. Indeed, numerous grazer species from wild and semi-natural systems are known to successfully, and more or less continuously, disperse seeds through either attachment to fur and hooves (epizoochory), or through abundant ingestion of seeds with the foliage they feed on (endozoochory; Couvreur *et al.* 2005). Observations of endozoochory go back a relatively long time (e.g. von Marilaun 1902; others in Ridley 1930), and now from temperate grasslands alone hundreds of plant species are known to show viable seeds in herbivore dung (e.g. Malo & Suarez 1995; Pakeman *et al.* 2002; Myers *et al.* 2004; Cosyns *et al.* 2005a; Mouissie *et al.* 2005a).

Through dispersal, vectors hold potential to select for particular seed traits and attributes (sensu Violle *et al.* 2007). For dispersal by e.g. birds, wind or ants, principal evolutionary-ecological underpinnings are relatively well-established, reflecting a thorough understanding of plant-vector interactions (e.g. Vander Wall 2001; Cheptou *et al.* 2008; Manzaneda *et al.* 2009). Drawing from observations of dung-borne seeds and from diverse theoretical considerations, Janzen (1984) was the first to suggest herbivorous mammals (grazers) as a selective force on seed attributes, too. Although his ultimate views of a widespread endozoochorous syndrome were soon countered by a lack of empirical support (Collins & Uno 1985), the idea that grazers can at least in some cases significantly exert selection, seemed launched and accepted (e.g. Quinn *et al.* 1994; Malo & Suarez 1995; Cousens *et al.* 2008). This might be premature, however, as our understanding of the seed-grazer interaction, a.o. the traits that function in the process and hence might be selected for, is still relatively poor.

Of the several stages involved in endozoochory (Wang & Smith 2002), seed mortality due to digestion is recognized as a severe bottleneck. Former feeding trials in which mortality rates were related to seed characteristics yielded contrasting results. Smaller-seeded species tended to show lower mortality rates than larger-seeded species in the feeding trial of Mouissie *et al.* (2005b), whereas the opposite was found by both Peco *et al.* (2006) and Cosyns *et al.* (2005b). For (their measures of) seed shape, a negative, neutral, and positive effect were found respectively.

At least a part of these inconsistencies might come from a statistical problem suffered by these analyses, obscuring interpretation and comparison. Basically, seed attributes are considered *across* species; its results can be extrapolated beyond the studied species only when they properly represent the composition of the targeted plant community. When one wants to interpret the relationship causally, however, caution is called for (Silvertown & Dodd 1996). Because species are related to each other through common descent, they cannot be regarded as independent data points, an essential for relevant statistical analysis (Garland *et al.* 2005). 'Comparative methods', evaluating functional relationships from species comparisons (Harvey & Pagel 1995), accordingly made progress in recent

decades by constructing several methods that control for interspecific affinities. Of these, the method of phylogenetically independent contrasts (PIC) was the first to be elaborated and to be widely applied (see Garland *et al.* 2005 for a review).

There is, moreover, still a lack of basic empirical data on the topic. Will & Tackenberg (2008) recently stressed the importance, but scarcity of plant species' gut passage data in their review, advocating further need of information.

We performed an experiment determining mortality rates of seeds fed to cattle for 48 plant species, the highest number included in any *in vivo* assay so far. Specifically, we aim (i) to contribute to the limited data of plant species performances in endozoochory, (ii) to reassess the role of simple seed traits using proper comparative-analytical tools, and (iii) to ultimately identify a system (i.e. a taxon and trait) that holds the potential to infer on endozoochory-imposed seed evolution.

METHODS

We assessed mortality rates by feeding known numbers of seeds to cattle. Note that, when referring to our numbers, we express them not as the percentage of seeds dying, i.e. mortality rate, but as the percentage of seeds surviving digestion ('survival rate'). This is because germination of ingested seeds is corrected for germination of non-ingested seeds, yielding a number that can exceed 100 % (when gut passage increases germination).

Species selection and seed attributes

Although they inherently do not represent wide ranges of seed attributes (see discussion), the selection was restricted to herbs and graminoids from open, mostly grassland(-like) habitats for several reasons. Firstly, these species can be considered the ones for which mortality rates are the most relevant, as they have good chances of being consumed on a regular basis. Secondly, we are concerned about the effects of small -rather than large- changes in seed attributes on mortality, given our objective to identify a trait with which the selective strength of endozoochory could be assessed. For such a trait, its relationship with mortality should also hold over small scales.

We obtained seeds of 48 plant species from commercial sources (Table 1). All are palatable and some of them have been found as viable seeds in herbivore dung from (semi)natural habitats (Cosyns *et al.* 2005a; Mouissie *et al.* 2005a). As defined above, in the text "seeds" refer to ecologically analogous structures (true seeds, fruits or mericarps) rather than morphologically homologous structures (Table 1).

Single-seed mass was assessed by weighing three samples of a known number of air-dried seeds (500 or 1000 seeds, 0.01 mg precision). Before measuring dimensions, we conducted manipulations in some species (Table 1): open and loose structures, such as glumes in grasses, perigynia in *Carex* and pericarps in *Geranium*, were removed. The assumption is that these readily disintegrating structures are unlikely to affect survival, while they would greatly bias trait measurements, and thus, correlations.

Dimensions (length, width and height) were measured on 10 randomly selected seeds per species using stereoscopic binoculars (25 µm precision). Seed length is defined as the longest of the three dimensions. Seed shape is expressed according to Thompson *et al.* (1993), as the variance in seed dimensions after dividing each dimension by length. We multiplied this measure by 100 for practicability.

Survival rate

Seed-to-feed quantities were measured using seed mass; for each species these quantities comprised about 5000 seeds per individual animal (due to limited supplies 3400 or 3000 for some). In a pack of 14 cows, tied individually in stable, each species was randomly attributed to seven animals, with the sole restriction of keeping congeneric plant species separate in order to facilitate later seedling identification. As such, an individual animal was fed between 21 and 27 species.

The animals were adult (three to four years old) cows of the Belgian Blue breed, weighing about 550-600 kg. Their standard diet mainly consisted of maize silage, pressed beet pulp, and hay; being a potential source of seed contamination, the latter was excluded about seven days before the experiment. One hour before feeding, dung samples were collected in order to (among others, see below) control for contamination.

The seeds were mixed through the silage and applied to all animals simultaneously during morning feeding. All dung was collected 6, 11, 24, 35, 48, 72, 96, 120 and 144 hours thereafter. Per collection the excrements were weighed and mixed for each individual animal. From these, samples were taken and weighed (± 475g). These samples were stratified for 34 days at ± 4.5°C, then mixed through a shallow sterile layer of potting soil-sand mixture (1:1) in trays (45x40x10cm), and left to germinate in a greenhouse for 8 months under a natural light regime (April-December 2007). Samples were watered manually.

As a control, we added seeds to dung samples that were collected one hour before feeding (non-ingested control; 100 seeds per species, 9-14 spp. per sample, again with congeneres kept separate, five samples per species). Apart from this, these samples experienced exactly the same treatments as the ones containing ingested seeds.

Per plant species and per individual animal, the survival rate was estimated as follows (Cosyns *et al.* 2005b),

$$\sum_{i=int1}^{i=int9} n_i \times (W_{Di} / W_{di}) \Big/ V * (W_{sb} / W_s) \qquad \text{(Eq. 1)}$$

where W_{Di} is the weight of the dung produced by the animal in the time interval passed. W_{di} is the weight of the dung sampled from it (± 475 g). The number of seedlings found germinating from this sample is denoted n_i. After doing the summation over all nine time intervals, the numerator yields an

estimation of the number of viable seeds excreted by the animal. As W_s is the weight of a single seed (assessed beforehand) and W_{sb} denotes the weight of the seed batch fed to the animal, W_{sb}/W_s equals the number of seeds fed to the animal (mostly ±5000). V is the mean proportion of seedlings germinating from the (five) control samples containing non-ingested seeds. Its insertion controls for the combined effect of intrinsic viability of the seeds and any possible effects of the dung environment on germination.

Data analysis

As noted in the introduction, multi-species analyses concerned with functional relationships should control for species interdependence. In the method of phylogenetic contrasts, this is done on the basis of a (phylogenetic) tree. Given the tree, i.e. given the ways in which species are related to each other, the method circumvents taxonomical bias by comparing ("contrasting") taxa in a pairwise manner both at the tips of the tree and at internal nodes. The contrasts themselves are independent from each other.

For the phylogeny of our species (Fig. 1A), we used the widely accepted APGII system (Stevens 2001 onwards) for both the assignment of genera to families and the topology of families. For two families additional sources were needed. We used the consensus trees published in Walker *et al.* (2004) and Quintanar *et al.* (2007) for the Lamiaceae and Poaceae, respectively. All branch lengths were arbitrarily set to 1.0 (unit).

Contrasts are calculated as elaborated in Felsenstein's (1985) fundamental paper. On these contrasts, linear regression is performed following the procedure set out by Garland *et al.* (1992), with intercept being zero. The unit branch lengths satisfy the conditions set by these authors. The three polytomies are considered soft, and the number of degrees of freedom in significance testing are lowered accordingly (42 d.f.; Purvis & Garland 1993). The analysis was executed using the PDAP module (Midford *et al.* 2005) implemented in Mesquite software (Maddison & Maddison 2009).

As contrasts are calculated on specific means, information is lost on intraspecific variation in seed survival. For each node of the tree, we tested for differences between both sides of the branching using standard permutation tests at the 0.10-level (2000 randomisations). These provide us with an idea of those contrasts that are most informative.

RESULTS

In total, 7453 seedlings emerged from the dung samples (holding either ingested or non-ingested seeds). Neither the sand-potting soil substrate, nor the dung samples themselves contained any relevant seed contamination; some did occur from the external environment, but all belonged to non-included taxa (e.g. *Oxalis*, *Epilobium*, *Betula*).

More than 90 % of the seeds was excreted within 48 hours after feeding and only a negligible fraction was found from the last time interval sampled (Fig. 2). No species-specific temporal patterns

were observed: nearly all of the species showed their maximal excretion from the 24- or 35-hour collection. The few exceptions had very few emerging seedlings, except for *Carex vulpina*, which showed a clear maximum from the 48-hour collection (data not shown). As retention parameters are considered pivotal in spatially explicit approaches of animal seed dispersal (e.g. Vellend *et al.* 2003; Will & Tackenberg 2008), results of a lognormal fit to these data are included in Fig. 2 (Rawsthorne *et al.* 2009).

Specific survival rates are shown in Fig. 1B. The exact numbers can be found as supplementary material, together with the germination rates from the non-ingested control. The species showing the highest proportions of seeds surviving ingestion were *Helianthemum nummularium*, *Trifolium pratense*, *T. campestre*, and *Juncus bufonius*. For the first two species mentioned, digestion even appeared to stimulate germination as survival rates exceeded 100 %. Remarkably, *Juncus effusus* did not germinate from the control, while it did germinate from the ingested seeds, though with low incidence (only two seedlings). This suggests a positive effect of ingestion on germination, too, but this cannot be formalized mathematically, since Eq. 1 in this case is a division by zero. It is therefore omitted from further analysis.

From the 19 congeneric species pairs, *Agrostis*, *Cardamine*, *Cerastium*, *Galium*, *Mentha*, *Myosotis*, *Potentilla* and *Thymus* yielded significant within-genus differences (permutation tests, 0.10-level). Except for *Agrostis* and *Myosotis*, in each pair one species showed zeros only.

Results of the seed mass, length, and shape measurements are included in Table 1.

There appeared no significant impact of each of the three studied seed traits (mass, length and shape) on seed survival rates of the species (Fig. 3). Within Fig. 3A, a point above the horizontal axis represents a contrast in which the heavier-seeded taxon of the pair showed the higher survival, whereas a point below the horizontal axis represents a contrast in which the lighter-seeded taxon performed better. Overall, the points counterbalance both in number and magnitude, and no overall effect is observed. The same reasoning applies to Fig. 3B and C.

DISCUSSION

The proportion of seeds surviving ingestion differed profoundly between species: most species show high mortality rates (up to 100 % of the seeds ingested) and a decreasing number of species show lower mortality rates (down to 0 %). Overall, this corresponds to patterns found by others (references below). Note that the increased germination rates observed in *Helianthemum nummularium* and *Trifolium pratense* should not be mistaken with mortality rates, which are essentially zero.

The absence of an effect of seed mass on mortality found here, supports some literature accounts (e.g. Gardener *et al.* 1993; Bruun & Poschlod 2006), but not others (Cosyns *et al.* 2005b; Mouissie *et al.* 2005b; Peco *et al.* 2006), and the same is true for seed shape. As noted in the introduction, however, cross-species analyses suffer problems which make comparison difficult. For instance, drawing on a correlation across mortality rates from 25 species fed to deer, Mouissie *et al.* (2005b) concluded seed

size to be positively related to mortality. When one applies a contrasting perspective however, e.g. by considering the pattern within the included Asteraceae or within the graminoids (the largest groups), the data clearly suggests no effect. This is supported by PIC analysis among all species, which thus refutes the author's conclusion. So, taxonomical interdependence clearly can affect analyses of relationships among these traits significantly.

A reflection could be made on the attribute ranges considered. In nature, seed size varies enormously, over 10 orders of magnitude (± 10^{-3}-10^{7} mg; Harper *et al.* 1970), with species from fields, pastures, forest clearings, and other habitats that are inviting to grazers comprising a subset hereof (± 10^{-2}-10^{1} mg; Salisbury 1942; Lord *et al.* 1995). From these wide ranges, it is up to the experimenter to choose which range is considered most relevant, with any claim of an "effect of size on mortality" restricted to that range. The studies mentioned above all stay in the lower range (± 10^{-2}-10^{2} mg). We cannot rule out size to have an effect beyond this range (10^{3} mg up to the maximal size animals are able to ingest) as wide-scale studies herein are lacking. We stuck to the lower range, too, on grounds stated in the introduction, although frequent ingestion of large seeds by grazers might be of relevance in the (sub)tropics (e.g. Janzen 1982).

If neither size nor shape show clear relationships with species' performances, which traits could do so? Reconsidering the three species performing best (Fig. 1B), they have one important seed attribute in common: they are the only species included known to have physically dormant seeds (Baskin & Baskin 2001), i.e. their seeds are initially water-impermeable. The favourable effect of this so-called 'hardseededness' is confirmed in several studies on dispersal of Fabaceae and Cistaceae by ungulates (Suckling 1952; Janzen 1981; Gardener *et al.* 1993; Malo & Suarez 1998; Ramos *et al.* 2006): impermeable seeds survive the digestive tract, permeable seeds do not. The observation of increased germination rates is then linked to a well-timed loss of dormancy (Baskin & Baskin 2001). If one considers this trait, the benefits of having a water-impermeable barrier are clear since, next to exposure to the chewing apparatus, it presumably are the digestive fluids - containing proteolytic enzymes and bacteria - that pose the severest threat to the seed. Accordingly, it is probable that other structural, anatomical, and/or textural traits (e.g. seed surface or seed coat attributes) are likely to overrule simple morphological traits such as size or shape in explaining mortality rate. Until this date, we still lack knowledge on the effect of suchlike attributes for dry-fruited herbs, whereas for fleshy-fruited shrubs and trees they are more often dealt with (e.g. in ornithochory; Traveset 1998; Traveset *et al.* 2008). When excluding the physically dormant species from our analyses, seed mass relates negatively to survival rate ($P = 0.015$). This is, however, due to the extreme values in *Juncus bufonius*; further exclusion of this species renders the relationship back to non-significant. Peco *et al.* (2006) found this species to have "highly water-impermeable seeds", thus making it functionally similar to the physically dormant species. Moreover, mortality rates are very likely to be the result of several traits acting together, which advocates a multivariate approach in analyses that aim at a full comprehension of the process. So far, we have no indications on what might have caused the significant intrageneric contrasts

observed in *Agrostis*, *Cardamine*, *Cerastium*, etc.

As outlined in the introduction, an ultimate objective could be to identify a seed trait that holds potential to infer on seed evolution through endozoochory. For this purpose neither size (weight or length) nor shape seem suitable since no univocal effects are observed, at least not on a small scale. Water-impermeability might prove a better candidate, since it satisfies three pivotal conditions: it closely relates to survival following ingestion (accounts mentioned above), it is known to hold intraspecific variation (Baskin & Baskin 2001), and it has at least some of its parameters genetically based (e.g. Nair *et al.* 2004; Boersma *et al.* 2007). If grazers can induce seed evolution, this might be the trait to focus on in future research.

ACKNOWLEDGEMENTS

We would sincerely like to thank the Flanders Marine Institute (VLIZ) for providing greenhouse accommodation, and Gerard Stroo for his cattle. Indispensable help came from Frank Broucke, Linda Stroo and Rein Brys. We are also grateful to both anonymous reviewers for their constructive comments on an earlier draft of this manuscript. Bram D'hondt has a grant supplied by the Research Foundation-Flanders (FWO).

REFERENCES

Baskin C.C., Baskin J.M. (2001) *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego.

Boersma J.G., Buirchell B.J., Sivasithamparam K., Yang H. (2007) Development of a PCR marker tightly linked to *mollis*, the gene that controls seed dormancy in *Lupinus angustifolius* L. *Plant Breeding*, **126**, 612-616.

Bruun H.H., Poschlod P. (2006) Why are small seeds dispersed through animal guts: large numbers or seed size per se? *Oikos*, **113**, 402-411.

Cheptou P.O., Carrue O., Rouifed S., Cantarel A. (2008) Rapid evolution of seed dispersal in an urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of Sciences of the USA*, **105**, 3796-3799.

Collins S.L., Uno G.E. (1985) Seed predation, seed dispersal, and disturbance in grasslands: a comment. *American Naturalist*, **125**, 866-872.

Cosyns E., Claerbout S., Lamoot I., Hoffmann M. (2005a) Endozoochorous seed dispersal by cattle and horse in a spatially heterogeneous landscape. *Plant Ecology*, **178**, 149-162.

Cosyns E., Delporte A., Lens L., Hoffmann M. (2005b) Germination success of temperate grassland species after passage through ungulate and rabbit guts. *Journal of Ecology*, **93**, 353-361.

Cousens R., Dytham C., Law R. (2008) *Dispersal in plants. A population perspective*. Oxford University Press, New York.

Couvreur M., Cosyns E., Hermy M., Hoffmann M. (2005) Complementarity of epi- and endozoochory of plant seeds by free ranging donkeys. *Ecography*, **28**, 37-48.

Felsenstein J. (1985) Phylogenies and the comparative method. *American Naturalist*, **125**, 1-15.

Gardener C.J., McIvor J.G., Jansen A. (1993) Passage of legume and grass seeds through the digestive tract of cattle and their survival in faeces. *Journal of Applied Ecology*, **30**, 63-74.

Garland T., Bennett A.F., Rezende E.L. (2005) Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology*, **208**, 3015-3035.

Garland T., Harvey P.H., Ives A.R. (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology*, **41**, 18-32.

Harper J.L., Lovell P.H., Moore K.G. (1970) The shapes and sizes of seeds. *Annual Review of Ecology and Systematics*, **1**, 327-356.

Harvey P.H., Pagel M.D. (1995) *The comparative method in evolutionary biology*. Oxford University Press, New York.

Janzen D.H. (1981) *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents. *Ecology*, **62**, 593-601.

Janzen D.H. (1982) Differential seed survival and passage rates in cows and horses, surrogate Pleistocene dispersal agents. *Oikos*, **38**, 150-156.

Janzen D.H. (1984) Dispersal of small seeds by big herbivores: foliage is the fruit. *American Naturalist*, **123**, 338-353.

Lord J., Westoby M., Leishman M. (1995) Seed size and phylogeny in six temperate floras: constraints, niche conservatism, and adaptation. *American Naturalist*, **146**, 349-364.

Maddison W.P., Maddison D.R. (2009) *Mesquite: a modular system for evolutionary analysis.* Version 2.6.

Malo J.E., Suarez F. (1995) Herbivorous mammals as seed dispersers in a Mediterranean dehesa. *Oecologia*, **104**, 246-255.

Malo J.E., Suarez F. (1998) The dispersal of a dry-fruited shrub by red deer in a Mediterranean ecosystem. *Ecography*, **21**, 204-211.

Manzaneda A.J., Rey P.J., Alcantara J.M. (2009) Conflicting selection on diaspore traits limits the evolutionary potential of seed dispersal by ants. *Journal of Evolutionary Biology*, **22**, 1407-1417.

Midford P.E., Garland Jr. T., Maddison W.P. (2005) *PDAP package of Mesquite.* Version 1.07.

Mouissie A.M., Van Der Veen C.E.J., Veen G.F., Van Diggelen R. (2005a) Ecological correlates of seed survival after ingestion by fallow deer. *Functional Ecology*, **19**, 284-290.

Mouissie A.M., Vos P., Verhagen H.M.C., Bakker J.P. (2005b) Endozoochory by free-ranging, large herbivores: ecological correlates and perspectives for restoration. *Basic and Applied Ecology*, **6**, 547-558.

Myers J.A., Vellend M., Gardescu S., Marks P.L. (2004) Seed dispersal by white-tailed deer: implications for long-distance dispersal, invasion, and migration of plants in eastern North America. *Oecologia*, **139**, 35-44.

Nair R.M., Craig A.D., Rowe T.D., Biggins S.R., Hunt C.H. (2004) Genetic variability and heritability estimates for hardseededness and flowering in balansa clover (*Trifolium michelianum* Savi) populations. *Euphytica*, **138**, 197-203.

Pakeman R.J., Digneffe G., Small J.L. (2002) Ecological correlates of endozoochory by herbivores. *Functional Ecology*, **16**, 296-304.

Peco B., Lopez-Merino L., Alvir M. (2006) Survival and germination of Mediterranean grassland species after simulated sheep ingestion: ecological correlates with seed traits. *Acta Oecologica*, **30**, 269-275.

Purvis A., Garland T. (1993) Polytomies in comparative analyses of continuous characters. *Systematic Biology*, **42**, 569-575.

Quinn J.A., Mowrey D.P., Emanuele S.M., Whalley R.D.B. (1994) The "Foliage is the Fruit" hypothesis: *Buchloe dactyloides* (Poaceae) and the shortgrass prairie of North America. *American Journal of Botany*, **81**, 1545-1554.

Quintanar A., Castroviejo S., Catalan P. (2007) Phylogeny of the tribe Aveneae (Pooideae, Poaceae) inferred from plastid trnT-F and nuclear ITS sequences. *American Journal of Botany*, **94**, 1554-1569.

Ramos M.E., Robles A.B., Castro J. (2006) Efficiency of endozoochorous seed dispersal in six dry-fruited species (Cistaceae): from seed ingestion to early seedling establishment. *Plant Ecology*, **185**, 97-106.

Rawsthorne J., Roshier D.A., Murphy S.R. (2009) A simple parametric method for reducing sample sizes in gut passage time trials. *Ecology*, **90**, 2328-2331.

Ridley H.N. (1930) *The dispersal of plants throughout the world*. L. Reeve & Co., LTD., Ashford.

Salisbury E.J. (1942) *The reproductive capacity of plants*. G. Bell & sons, London.

Silvertown J., Dodd M. (1996) Comparing plants and connecting traits. *Philosophical Transactions of the Royal Society of London B*, **351**, 1233-1239.

Stevens, P. F. (2001 onwards) Angiosperm phylogeny website.
http://www.mobot.org/MOBOT/research/APweb/

Suckling F.E.T. (1952) Dissemination of white clover (*Trifolium repens*) by sheep. *New Zealand Journal of Science and Technology*, **33**, 64-77.

Thompson K., Band S.R., Hodgson J.G. (1993) Seed size and shape predict persistence in soil. *Functional Ecology*, **7**, 236-241.

Traveset A., Rodriguez-Perez J., Pias B. (2008) Seed trait changes in dispersers' guts and consequences for germination and seedling growth. *Ecology*, **89**, 95-106.

Traveset A. (1998) Effect of seed passage through vertebrate frugivores' guts on germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics*, **1/2**, 151-190.

Vander Wall S.B. (2001) The evolutionary ecology of nut dispersal. *The Botanical Review*, **67**, 74-117.

Vellend M., Myers J.A., Gardescu S., Marks P.L. (2003) Dispersal of *Trillium* seeds by deer: implications for long-distance migration of forest herbs. *Ecology*, **84**, 1067-1072.

Violle C., Navas M.-L., Vile D., Kazakou E., Fortunel C., Hummel I., Garnier E. (2007) Let the concept of trait be functional! *Oikos*, **116**, 882-892.

von Marilaun A.K. (1902) *The natural history of plants. Their forms, growth, reproduction and distribution.* Blackie & Son, London.

Walker J.B., Sytsma K.J., Treutlein J., Wink M. (2004) *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany*, **91**, 1115-1125.

Wang B.C., Smith T.B. (2002) Closing the seed dispersal loop. *Trends in Ecology & Evolution*, **17**, 379-385.

Will H., Tackenberg O. (2008) A mechanistic simulation model of seed dispersal by animals. *Journal of Ecology*, **96**, 1011-1022.

Table 1. Included species (with abbreviations, cf. Fig. 1), propagules (prop.), and their measures (mean and standard error; n = 3 for mass, n = 10 for shape and length). S, seed; F, fruit ([1]without exocarp); Mc, mericarp. Propagules marked with an asterisk were manipulated before measuring length and shape (see text for details).

species name	abb.	prop.	mass [mg]	length [µm]	shape
Achillea millefolium L.	Ach mil	F	0.122 (0.001)	1590 (57)	12.60 (0.25)
Achillea ptarmica L.	Ach pta	F	0.233 (0.006)	1680 (53)	9.96 (0.22)
Agrostis capillaris L.	Agr cap	F*	0.069 (0.003)	905 (24)	8.65 (0.31)
Agrostis stolonifera L.	Agr sto	F*	0.122 (0.001)	1068 (25)	8.74 (0.19)
Artemisia campestris L.	Art cam	F	0.090 (0.004)	1028 (41)	8.25 (0.38)
Artemisia vulgaris L.	Art vul	F	0.136 (0.001)	1415 (44)	10.92 (0.18)
Briza media L.	Bri med	F*	0.885 (0.007)	1498 (43)	6.58 (0.23)
Briza minor L.	Bri min	F*	0.838 (0.024)	1510 (31)	5.35 (0.35)
Campanula rotundifolia L.	Cam rot	S	0.079 (0.000)	848 (20)	5.74 (0.27)
Cardamine hirsuta L.	Car hir	S	0.116 (0.000)	958 (24)	9.09 (0.62)
Cardamine pratensis L.	Car pra	S	0.419 (0.004)	1483 (53)	7.00 (0.49)
Carex flacca Schreb.	Car fla	F*	0.805 (0.013)	1540 (37)	2.35 (0.16)
Carex vulpina L.	Car vul	F*	1.495 (0.006)	2070 (43)	7.89 (0.23)
Cerastium dubium (Bast.) Guépin	Cer dub	S	0.095 (0.000)	660 (18)	2.52 (0.24)
Cerastium fontanum Baumg.	Cer fon	S	0.123 (0.001)	745 (14)	3.53 (0.30)
Chenopodium album L.	Che alb	F[1],*	0.616 (0.003)	1200 (33)	4.27 (0.22)
Chenopodium bonus-henricus L.	Che bon	F[1],*	1.876 (0.014)	1670 (43)	2.63 (0.15)
Galium aparine L.	Gal apa	Mc	4.705 (0.011)	2333 (53)	1.72 (0.17)
Galium verum L.	Gal ver	Mc	0.349 (0.002)	1040 (40)	2.21 (0.25)
Geranium molle L.	Ger mol	S*	1.265 (0.033)	1485 (32)	2.38 (0.21)
Geranium robertianum L.	Ger rob	S*	1.701 (0.008)	1908 (28)	4.74 (0.19)
Helianthemum nummularium (L.) Mill.	Hel num	S	0.961 (0.011)	1573 (39)	4.14 (0.43)
Hypericum perforatum L.	Hyp per	S	0.131 (0.000)	1160 (15)	7.83 (0.18)
Hypericum tetrapterum Fries	Hyp tet	S	0.037 (0.000)	750 (27)	7.46 (0.38)
Juncus bufonius L.	Jun buf	S	0.022 (0.001)	413 (9)	2.17 (0.14)
Juncus effusus L.	-	S	0.017 (0.001)	463 (24)	5.88 (0.45)
Mentha aquatica L.	Men aqu	Mc	0.141 (0.003)	890 (12)	2.49 (0.15)
Mentha pulegium L.	Men pul	Mc	0.089 (0.000)	675 (19)	2.85 (0.19)
Myosotis arvensis (L.) Hill	Myo arv	Mc	0.296 (0.001)	1405 (28)	5.23 (0.13)
Myosotis scorpioides L.	Myo sco	Mc	0.437 (0.001)	1603 (69)	4.78 (0.33)
Origanum majorana L.	Ori maj	Mc	0.236 (0.001)	1013 (18)	3.61 (0.18)
Origanum vulgare L.	Ori vul	Mc	0.077 (0.000)	765 (19)	4.28 (0.23)
Pimpinella saxifraga L.	Pim sax	Mc	0.396 (0.003)	1480 (58)	6.53 (0.31)
Plantago lanceolata L.	Pla lan	S	2.094 (0.027)	2780 (66)	9.24 (0.23)
Poa annua L.	Poa ann	F*	0.382 (0.008)	1383 (31)	6.34 (0.37)
Poa pratensis L.	Poa pra	F*	0.320 (0.006)	1538 (52)	9.43 (0.27)
Potentilla argentea L.	Pot arg	F	0.135 (0.002)	1040 (10)	4.32 (0.19)
Potentilla tabernaemontani Aschers.	Pot tab	F	0.581 (0.007)	1568 (30)	4.63 (0.09)
Primula vulgaris Huds.	Pri vul	S	0.975 (0.002)	1483 (62)	2.88 (0.39)
Ranunculus bulbosus L.	Ran bul	F	3.146 (0.039)	2995 (63)	8.22 (0.25)
Rumex acetosa L.	Rum ace	F	0.883 (0.017)	1988 (41)	4.43 (0.31)
Rumex obtusifolius L.	Rum obt	F	1.595 (0.015)	2108 (68)	3.83 (0.25)
Silene vulgaris (Moench) Garcke	Sil vul	S	0.949 (0.021)	1380 (42)	2.56 (0.25)
Thymus pulegioides L.	Thy pul	Mc	0.131 (0.003)	693 (24)	1.62 (0.17)
Thymus serpyllum L.	Thy ser	Mc	0.122 (0.001)	713 (12)	1.52 (0.17)
Trifolium campestre Schreb.	Tri cam	S	0.668 (0.010)	1125 (45)	4.91 (0.46)
Trifolium pratense L.	Tri pra	S	1.385 (0.009)	1730 (60)	3.75 (0.26)
Viola arvensis Murray	Vio arv	S	0.684 (0.006)	1718 (24)	5.35 (0.19)

FIGURES

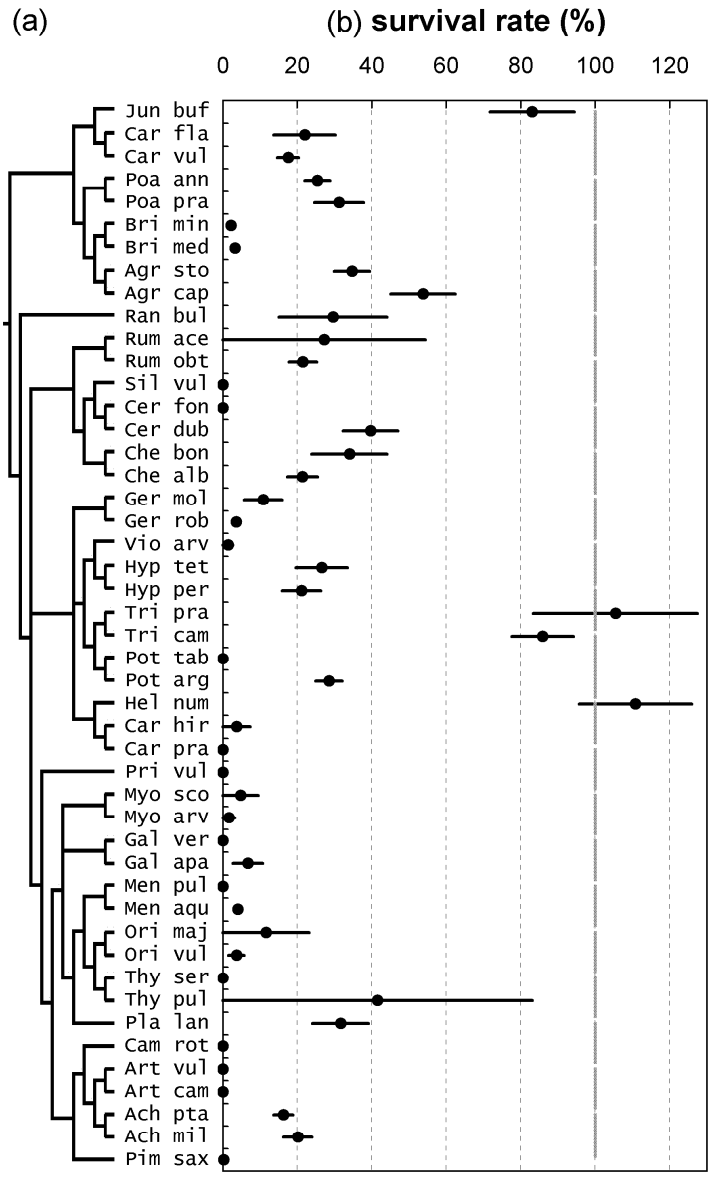

Fig. 1. A: Working phylogeny of the species. Branches are not drawn to scale. B: Mean (± S.E.) proportion of seeds surviving ingestion by cattle, calculated as the germination rate of ingested seeds corrected for that of non-ingested seeds sown on the same substrate. Species names are abbreviated (see Table 1).

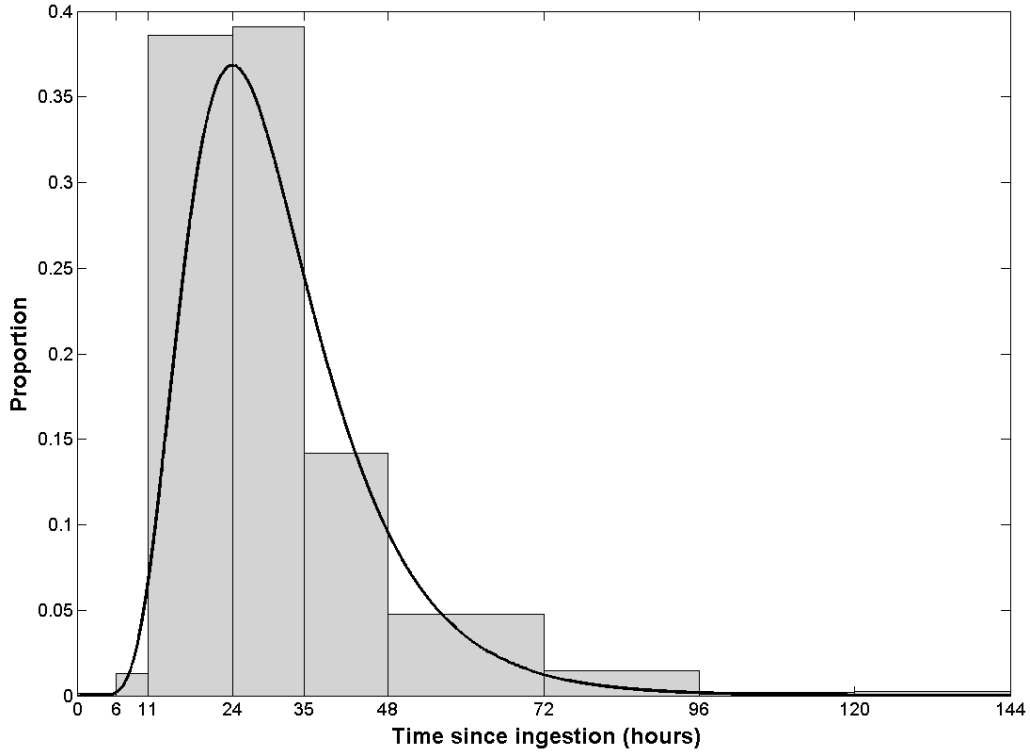

Fig. 2. Excretion of seeds through time. The bars represent the experimentally assessed proportions of seeds excreted. All species are pooled. A lognormal curve is fitted to these data (Rawsthorne *et al.* 2009); the parameters are $\mu = 3.35$ and $\sigma = 0.42$.

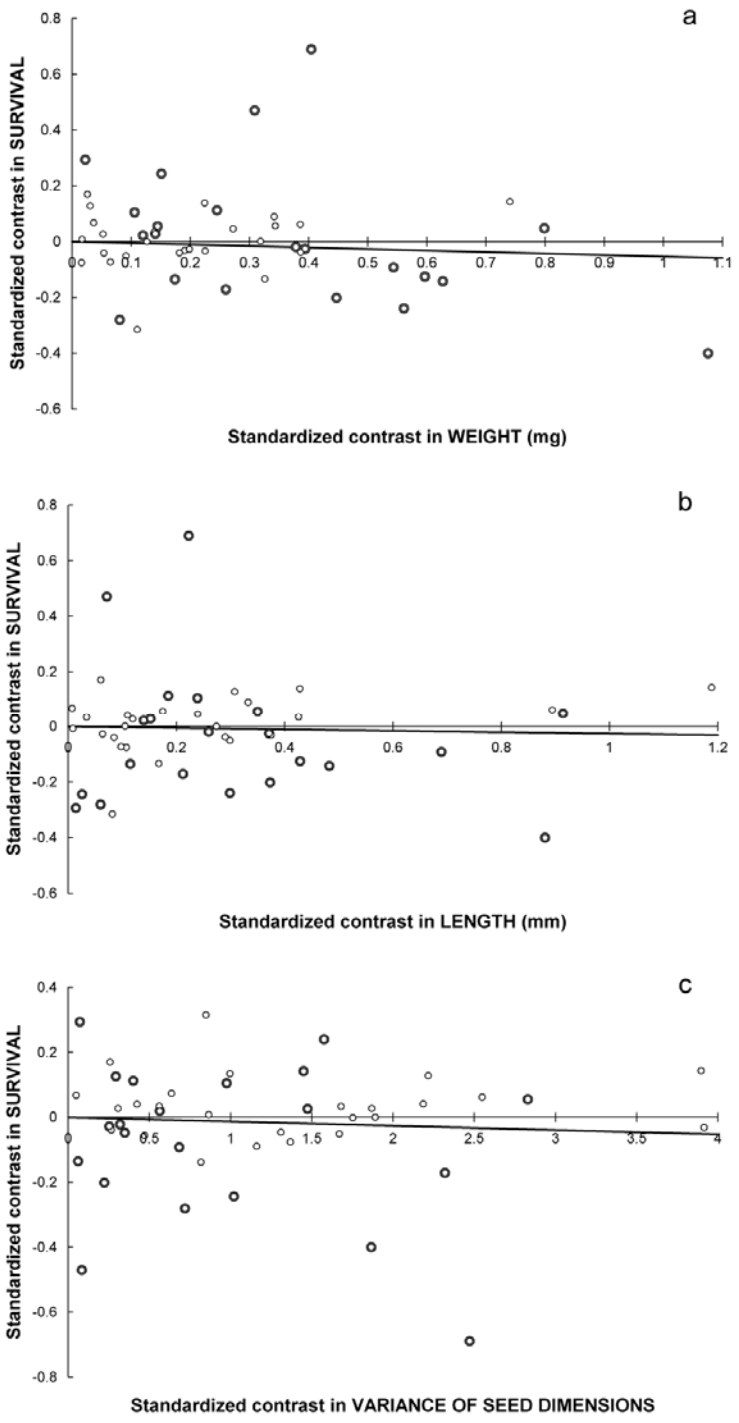

Fig. 3. Bivariate scatterplot of standardized independent contrasts in seed survival rate and log-transformed seed weight (A), seed length (B), and seed shape (C). Statistics are from least squares regressions (through origin, Garland *et al.* 1992; number of contrasts = 46, degrees of freedom = 42). Contrasts that proved significant in the permutation tests are bold-margined.

SUPPLEMENT

species name	control mean	s.e.	survival rate mean	s.e.
Achillea millefolium L.	55.20	1.28	20.12	3.86
Achillea ptarmica L.	48.40	3.14	16.22	2.61
Agrostis capillaris L.	50.20	5.89	53.78	8.67
Agrostis stolonifera L.	69.40	2.36	34.67	4.75
Artemisia campestris L.	1.60	0.40	0.00	0.00
Artemisia vulgaris L.	1.60	0.51	0.00	0.00
Briza media L.	61.60	4.17	3.29	1.14
Briza minor L.	47.20	2.44	2.17	0.48
Campanula rotundifolia L.	5.40	1.36	0.00	0.00
Cardamine hirsuta L.	2.40	1.17	3.67	3.67
Cardamine pratensis L.	4.40	1.75	0.00	0.00
Carex flacca Schreb.	1.60	0.51	22.00	8.29
Carex vulpina L.	17.00	4.44	17.54	2.86
Cerastium dubium (Bast.) Guépin	11.20	0.49	39.69	7.37
Cerastium fontanum Baumg.	0.60	0.40	0.00	0.00
Chenopodium album L.	13.80	3.06	21.36	4.06
Chenopodium bonus-henricus L.	6.40	2.01	34.01	10.13
Galium aparine L.	7.40	1.69	6.74	4.05
Galium verum L.	3.40	0.98	0.00	0.00
Geranium molle L.	3.60	1.50	10.80	5.09
Geranium robertianum L.	22.20	4.19	3.58	0.59
Helianthemum nummularium (L.) Mill.	5.40	1.50	110.85	15.15
Hypericum perforatum L.	5.80	1.46	21.13	5.22
Hypericum tetrapterum Fries	17.20	2.63	26.60	6.94
Juncus bufonius L.	8.40	2.23	83.07	11.33
Juncus effusus L.	0.00	0.00	n/a*	n/a*
Mentha aquatica L.	25.80	4.79	4.03	1.02
Mentha pulegium L.	1.20	0.58	0.00	0.00
Myosotis arvensis (L.) Hill	3.60	1.17	1.63	1.63
Myosotis scorpioides L.	1.80	0.49	4.77	4.77
Origanum majorana L.	0.20	0.20	11.60	11.60
Origanum vulgare L.	7.00	1.45	3.65	2.10
Pimpinella saxifraga L.	10.40	1.99	0.18	0.18
Plantago lanceolata L.	45.60	5.41	31.64	7.53
Poa annua L.	60.20	3.69	25.42	3.39
Poa pratensis L.	52.80	2.67	31.26	6.62
Potentilla argentea L.	14.40	2.87	28.50	3.58
Potentilla tabernaemontani Aschers.	0.60	0.40	0.00	0.00
Primula vulgaris Huds.	0.20	0.20	0.00	0.00
Ranunculus bulbosus L.	0.60	0.40	29.57	14.51
Rumex acetosa L.	0.20	0.20	27.19	27.19
Rumex obtusifolius L.	65.00	2.21	21.49	3.74
Silene vulgaris (Moench) Garcke	0.20	0.20	0.00	0.00
Thymus pulegioides L.	0.20	0.20	41.56	41.56
Thymus serpyllum L.	0.40	0.24	0.00	0.00
Trifolium campestre Schreb.	17.80	1.53	85.92	8.28
Trifolium pratense L.	2.80	0.86	105.48	22.07
Viola arvensis Murray	7.20	2.15	1.36	1.36

* Since the control germination rate equals zero, the survival rate estimate is undefined in this species. However, 2 seedlings emerged from the dung samples holding ingested seeds.